


# T cell migration assays

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 An abbreviated version of this protocol was published in Science Advances in Jan 2021

Tumor immunological phenotype signature-based high-throughput screening for the discovery of combination immunotherapy compounds

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## Detailed protocol

### T cell migration assays

Human peripheral blood-derived mononuclear cells were isolated from donor by centrifugation on a Ficoll-Hypaque gradient, washed with Hank's balanced salt solution and suspended in RPMI-1640 complete media (with 100 µg/ml streptomycin). Peripheral blood-derived mononuclear cells were activated for 2 days with 5 µg/ml anti-human CD3 Ab (eBiosciences) and 2 µg/ml anti-CD28 Ab (eBiosciences), then expanded for 5 days to generate activated T cells with recombinant human IL-2 (Novartis). In vitro migration assays were performed in a Transwell system (Corning) with a polycarbonate membrane of 6.5-mm diameter with a 3 µm pore size. These activated T cells were loaded into the top chamber of transwell inserts. In the bottom well, RPMI-1640 medium containing culture supernatant from MDA-MB-231 cells with ENMD-2076 treatment (5 µM) for 48 hour was added. Plates were incubated at 37 °C for 2-6 h; the contents of the lower chamber were collected; and the percentage of CD8+ T cells present in the bottom chamber was determined by FACS (LSR II, BD Biosciences).

**How to cite:** (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Wang, D. (2021). T cell migration assays. Bio-protocol Preprint. [bio-protocol.org/prep1091](https://bio-protocol.org/prep1091).
2. Wang, H., Li, S., Wang, Q., Jin, Z., Shao, W., Gao, Y., Li, L., Lin, K., Zhu, L., Wang, H., Liao, X. and Wang, D. (2021). Tumor immunological phenotype signature-based high-throughput screening for the discovery of combination immunotherapy compounds. Science Advances 7(4). DOI: [10.1126/sciadv.abd7851](https://doi.org/10.1126/sciadv.abd7851)

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